CLAIMS

We claim:

1. A method of detecting the presence of a target RNA molecule by detecting non-target cleavage products comprising:

a) providing:

i) a cleavage means,

ii) a source of target RNA, said target RNA having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

iii) a first oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said first oligonucleotide contains a sequence complementary to said second region of said target RNA and wherein said 3' portion of said first oligonucleotide contains a sequence complementary to said third region of said target RNA;

iv) a second oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said first region of said target RNA and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid;

b) mixing said cleavage means, said target RNA, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said 3' portion of said first oligonucleotide is annealed to said target RNA and wherein at least said 5' portion of said second oligonucleotide is annealed to said target RNA so as to create a cleavage structure and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products; and

c) detecting said non-target cleavage products.

2. The method of Claim 1 wherein said reaction conditions comprise a cleavage reaction temperature which is less than the melting temperature of said first oligonucleotide when annealed to said target RNA and greater than the melting temperature of said 3' portion of said first oligonucleotide.

3. The method of Claim 1 wherein said reaction temperature is between approximately 40 and 65 degrees centigrade.

4. The method of Claim 1 wherein said first and second oligonucleotides comprise DNA.

5. The method of Claim 1 wherein said cleavage means comprises a thermostable 5' nuclease.

6. The method of Claim 5 wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

7. The method of Claim 6 wherein said organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

8. The method of Claim 7 wherein said nuclease is encoded by a DNA sequence selected from the group consisting of SEQ ID NOS:1-3, 9, 10, 12, 21, 30 and 31.

9. The method of Claim 1 wherein said first oligonucleotide is completely complementary to said target RNA and wherein said second oligonucleotide is completely complementary to said target RNA.

10. The method of Claim 1 wherein said first oligonucleotide is partially complementary to said target RNA.

11. The method of Claim 1 wherein said second oligonucleotide is partially complementary to said target RNA.

12. The method of Claim 1 wherein said detection of said non-target cleavage products comprises electrophoretic separation of the products of said reaction followed by visualization of said separated non-target cleavage products.

13. The method of Claim 1 wherein said source of target RNA comprises a sample selected from the group comprising blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and semen.

14. The method of Claim 1 wherein said reaction conditions comprise providing a source of divalent cations.

15. The method of Claim 14 wherein said divalent cation is selected from the group comprising Mn^{2+} and Mg^{2+} ions.

16. A method of separating nucleic acid molecules, comprising:

a) providing:

i) a charge-balanced oligonucleotide and

ii) a reactant;

b) mixing said charge-balanced oligonucleotide with said reactant to create a reaction mixture under conditions such that a charge-unbalanced oligonucleotide is produced; and

c) separating said charge-unbalanced oligonucleotide from said reaction mixture.

17. The method of Claim 16, wherein said reactant comprises a cleavage means.

18. The method of Claim 17, wherein said cleavage means is an endonuclease.

19. The method of Claim 17, wherein said cleavage means is an exonuclease.

20. The method of Claim 16, wherein said reactant comprises a polymerization means.

21. The method of Claim 16, wherein said reactant comprises a ligation means.

22. The method of Claim 16, wherein said charge-balanced oligonucleotide comprises a label.

23. The method of Claim 16, wherein said charge-balanced oligonucleotide comprises one or more phosphonate groups.

24. The method of Claim 16, wherein said charge-balanced oligonucleotide has a net neutral charge and said charge-unbalanced oligonucleotide has a net positive charge.

25. The method of Claim 16, wherein said charge-balanced oligonucleotide has a net neutral charge and said charge-unbalanced oligonucleotide has a net negative charge.

26. The method of Claim 16, wherein said charge-balanced oligonucleotide has a net negative charge and said charge-unbalanced oligonucleotide has a net positive charge.

27. The method of Claim 16, wherein said charge-balanced oligonucleotide has a net negative charge and said charge-unbalanced oligonucleotide has a net neutral charge.

28. The method of Claim 16, wherein said charge-balanced oligonucleotide has a net positive charge and said charge-unbalanced oligonucleotide has a net neutral charge.

29. The method of Claim 16, wherein said charge-balanced oligonucleotide has a net positive charge and said charge-unbalanced oligonucleotide has a net negative charge.

30. The method of Claim 17, wherein said charge-balanced oligonucleotide comprises DNA containing one or more positively charged adducts.

31. The method of Claim 30, wherein said cleavage means removes one or more nucleotides from said charge-balanced oligonucleotide to produce said charge-unbalanced oligonucleotide, wherein said charge-unbalanced oligonucleotide has a net positive charge.

32. The method of Claim 30, wherein said cleavage means removes one or more nucleotides from said charge-balanced oligonucleotide to produce said charge-unbalanced oligonucleotide, wherein said charge-unbalanced oligonucleotide has a net neutral charge.

33. The method of Claim 30, wherein said cleavage means removes one or more nucleotides from said charge-balanced oligonucleotide to produce said charge-unbalanced oligonucleotide, wherein said charge-unbalanced oligonucleotide has a net negative charge.

34. The method of Claim 17, wherein said charge-balanced oligonucleotide comprises DNA containing one or more negatively charged adducts.

35. The method of Claim 34, wherein said cleavage means removes one or more nucleotides from said charge-balanced oligonucleotide to produce said charge-unbalanced oligonucleotide, wherein said charge-unbalanced oligonucleotide has a net negative charge.

36. The method of Claim 34, wherein said cleavage means removes one or more nucleotides from said charge-balanced oligonucleotide to produce said charge-unbalanced oligonucleotide, wherein said charge-unbalanced oligonucleotide has a net neutral charge.

37. The method of Claim 34, wherein said cleavage means removes one or more nucleotides from said charge-balanced oligonucleotide to produce said charge-unbalanced oligonucleotide, wherein said charge-unbalanced oligonucleotide has a net negative charge.

38. The method of Claim 30, wherein said one or more positively charged adducts are selected from the group consisting of indodicarbocyanine dye amidites, amino-substituted nucleotides, ethidium bromide, ethidium homodimer, (1,3-propanediamino)propidium, (diethylenetriamino)propidium, thiazole orange, (N-N'-tetramethyl-1,3-propanediamino)propyl thiazole orange, (N-N'-tetramethyl-1,2-ethanediamino)propyl thiazole orange, thiazole orange-thiazole orange homodimer (TOTO), thiazole orande-thiazole blue heterodimer (TOTAB), thiazole orange-ethidium heterodimer 1 (TOED1), thiazole orange-ethidium heterodimer 2 (TOED2) and florescien-ethidium heterodimer (FED).

39. The method of Claim 16, wherein said separating comprises subjecting said reaction mixture to an electrical field comprising a positive pole and a negative pole under conditions such that said charge-unbalanced oligonucleotide migrates toward said positive pole.

40. The method of Claim 16, wherein said separating comprises subjecting said reaction mixture to an electrical field comprising a positive pole and a negative pole under conditions such that said charge-unbalanced oligonucleotide migrates toward said negative pole.

41. The method of Claim 39 further comprising detecting the presence of said separated charge-unbalanced oligonucleotide.

42. A method of detecting cleaved nucleic molecules, comprising:

a) providing:

i) a homogeneous plurality of charge-balanced oligonucleotides;

ii) a sample suspected of containing a target nucleic acid having a sequence comprising a first region complementary to said charge-balanced oligonucleotide;

iii) a cleavage means; and

iv) a reaction vessel;

b) adding to said vessel, in any order, said sample, said charge-balanced oligonucleotides and said cleavage means to create a reaction mixture under conditions such that a portion of said charge-balanced oligonucleotides binds to said complementary target nucleic acid to create a bound population, and such that said cleavage means cleaves at least a portion of said bound population of charge-balanced oligonucleotides to produce a population of unbound, charge-unbalanced oligonucleotides; and

c) separating said unbound, charge-unbalanced oligonucleotides from sid reaction mixture.

43. The method of Claim 42 further comprising providing a homogeneous plurality of oligonucleotides complementary to a second region of said target nucleic acid, wherein said oligonucleotides are capable of binding to said target nucleic acid upstream of said charge-balanced oligonucleotides.

44. The method of Claim 43, wherein said first and said second region of said target nucleic acid share a region of overlap.

45. The method of Claim 42, wherein said cleavage means comprises a thermostable 5' nuclease.

46. The method of Claim 45 wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

47. The method of Claim 46 wherein said organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

48. The method of Claim 47 wherein said nuclease is encoded by a DNA sequence selected from the group consisting of SEQ ID NOS:1-3, 9, 10, 12, 21, 30 and 31.

49. The method of Claim 42, wherein said target nucleic acid comprises single-stranded DNA.

50. The method of Claim 42 wherein said target nucleic acid comprises double-stranded DNA and prior to the addition of said cleavage means said reaction mixture is treated such that said double-stranded DNA is rendered substantially single-stranded.

51. The method of Claim 50 wherein said treatment to render said double-stranded DNA is rendered substantially single-stranded by increasing the temperature.

52. The method of Claim 42 wherein said target nucleic acid comprises RNA.